ONLINE SUPPLEMENT 1

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ABBREVIATIONS

°C	degree Celsius (unit)
°F	degree Fahrenheit (unit)
A	ampere (unit)
ACLS	advanced cardiac life support
ASCDefib	name of one intervention group
ATP	adenosine triphosphate
bw	body weight
C	coulomb (unit)
CA	cardiac arrest
CONVDefib	name of one intervention group
CPR	cardiopulmonary resuscitation
Defib	defibrillation
F	French scale unit
g	gram
h	hour (unit)
Hz	hertz (unit)
ICD	implantable cardioverter defibrillators
L	liter (unit)
m	meter (unit)
min	minute (unit)
n. s.	not significant
OHCA	out of hospital cardiac arrest
Ω	ohm (unit)
ROC curve	receiver operating characteristic curve
ROSC	return/restoration of spontaneous circulation
s	second (unit)
V	volt (unit)
VF	ventricular fibrillation
VT	ventricular tachycardia

EXPANDED METHODS

We conducted a prospective, randomized, controlled, preclinical trial in 57 swine. Our investigation was approved by the animal welfare authority (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, reference number 84-02.04.2017.A176). Animals were managed in accordance with European and German laws on animal protection, the National Research Council Guide for the Care and Use of Laboratory Animals [1] and, more species-specific, the Guide for the Care and Use of Agricultural Animals in Research and Teaching [2]. Our reporting follows the ARRIVE guidelines [3] and the Utstein-style guidelines for laboratory research on CPR [4].

A computer-based complete randomization [5] of 52 swine to one of the two intervention groups ASCDefib or CONVDefib was performed. Except for one person not involved in data analysis operating the prototype defibrillator, any other investigator was blinded for the assignment. Anesthesia or surgery related effects were identified using another 5 sham-operated animals that received identical treatment but neither cardiac arrest nor cardiopulmonary resuscitation.

Medical Engineering

To perform this study in a blinded manner, corpuls | GS Elektromedizinische Geräte G. Stemple GmbH realized a prototype defibrillator with the ability to deliver both biphasic waveforms without the need for replugging with the following specifications: We aimed both waveforms to deliver the same amount of electrical energy. Following the current based approach [6] we chose fixed target values for first phase mean current with voltage being the dependant variable. In order to investigate predictive variables of successful defibrillation in electrical properties, we induced variation in the target values for first phase mean current by an approach comparable to commercially available defibrillators: In a subgroup of two thirds of all animals we set general target values for first phase mean current to 22.5 ampere (A) for ascending and 23.4 A for rectangular defibrillation waveforms. If the pre-shock measurement of transthoracic impedance exceeded 50 Ω these target values were reduced. In the remaining animals we used fixed target values of 24.5 A for ascending and 26.4 A for rectangular defibrillation waveforms without reducing first phase mean current in reaction to higher impedance.

The two different waveforms were similarly designed as 10 ms impulses with 6 ms and 4 ms for first and second phase, respectively. About 85 % of the total energy was allocated to the first phase of both biphasic waveforms. Prior to each defibrillation, the required voltage was determined by a 32 kHz alternating current measurement of interelectrode resistance to meet the set energy value. During each defibrillation, a microcontroller regulated voltage on the fly to readjust for impedance changes. For further investigation, we sampled current and voltage measured within the circuit at 4 kHz.

Since true ascending exponential waveforms with a slope near to infinity at their trailing edge would require enormous capacitors in transthoracic defibrillation, we chose to investigate a more ramp-shaped ascending waveform with a slope at about 3.7 $A \cdot ms^{-1}$. In assuming a membrane time constant of 3.5 ms, a first phase ascending ramp waveform of 6 ms (~1.7 time constants) delivers amounts of energy and maximum shock magnitude that are roughly comparable to ascending exponential waveforms according to Fishler's predictions. While a rectangular waveform follows mainly horizontally the prespecified target value for mean current, we designed the ascending biphasic waveform to exceed mean current approximately in the middle of the first phase.

To avoid leaving a residual charge on the myocardium [7], the second phase of the ascending waveforms decreases in current and voltage over time. The second phase of the rectangular waveforms was designed in a rectangular shape in the style of the known "corpuls bi-phasic" impulse. Figure 1 shows current over time [A, B] as well as voltage over time normalized to maximum voltage and the modeled membrane response according to the simplified resistor capacitor model [8] [C, D] for ascending and rectangular waveforms assuming that membrane time constant is 3.5 ms and ignoring voltage drops.

Pregelled self-adhesive defibrillation electrodes (corPatch easy, Leonhard Lang GmbH, Innsbruck, Austria, one attached to the right of the upper sternum, the apical one at the left postero-lateral chest wall) were connected to the prototype defibrillator.

Animal Preparation

We used healthy F1 hybrids German Landrace × Piétrain of both sexes with bodyweight (bw) 39.0 [37.5; 41.5] kg (age: about 9 weeks) because their reactions to ischaemia-reperfusion injury is highly comparable to humans. All swine were delivered by an agricultural breeder at least ten days before conduction of the experiments to our large animal facility, where they were kept in groups of up to 5 swine per pen. Ambient temperature was set to 293 K (68 °F, 20 °C) on a 12/12 hour (h) light/dark cycle, and straw bedded pens (9.3 m^2, > 1.85 m^2 per animal) were provided. Animals had ad libitum access to water and were fed twice a day with adequate nutrition. All animals were inspected at least once a day by animal keepers and veterinarians.

Prior to our experiments animals were fasted overnight with free access to water and separated from the group while maintaining visual contact. After adequate sedation depth had been reached by intramuscular nuchal injection of both azaperon (2.0 mg/kg bw, Stresnil, Janssen, Neuss, Germany) and midazolam (1.0 mg/kg bw, Midazolam 5 mg/ml, B. Braun, Melsungen, Germany), we oxygenated all swine on the way to the operating room to prevent hypoxemia. Under continuous monitoring of electrocardiogram (ECG) and pulse oximetry using a corpuls3 (corpuls, Kaufering, Germany) we completed general anesthesia by titrated intravenous injection of propofol (up to 10 mg/kg bw, Propofol 10 mg/ml MCT, Fresenius Kabi, Bad Homburg, Germany) and sufentanil (up to 0.6 µg/kg bw, Sufentanil-hameln, diluted to 10 µg/ml, hameln pharma plus, Hameln, Germany) via an ear vein catheter and by orotracheal

intubation in supine position. Throughout the whole experimentation, including euthanasia, we titrated total intravenous anesthesia in order to maintain the stage of surgical anesthesia in which swine did not respond to painful stimuli. For this purpose, we applied propofol (4.0 to 6.0 mg/kg bw/h, Propofol 20 mg/ml MCT, Fresenius Kabi, Bad Homburg, Germany), sufentanil (0.5 to 2.5 µg/kg bw/h) and midazolam if needed (up to 1.2 mg/kg bw/h). We provided Ringer's acetate solution (Jonosteril, Fresenius Kabi) at a rate of 10 ml/kg bw/h via a volumetric infusion pump (Infusomat fmS, B. Braun) throughout preparation, stabilization and experimentation.

After intubation, all swine were mechanically ventilated using a Fabius GS (Drägerwerk AG & Co. KGaA, Luebeck, Germany) with the following settings: volume-controlled, tidal volume 8 ml/kg bw, positive end-expiratory pressure (PEEP) 8 cmH_2O, fraction of inspired oxygen (F_iO_2) as low as possible to reach a peripheral oxygen saturation (S_pO_2) of at least 94%, and respiratory rate depending on endtidal carbon dioxide partial pressure ($etCO_2$).

For arterial blood pressure and cardiac output measurement we inserted a saline-filled catheter percutaneously into one femoral artery with its tip reaching the aorta (external diameter 1.33 mm (4 French, F), length 16 cm, PiCCO thermodilution catheter PV 2014L16 with blood pressure transducer from monitoring kit PV 8115, Getinge, Rastatt, Germany). The arterial pressure signal was processed by a PiCCOplus monitor (PULSION Medical Systems SE, Feldkirchen, Germany), sampled using PiCCO-VolEF Data Acquisition software V6.0.0 with a frequency of 1 Hz, and looped through to the corpuls3 monitor. We inserted a central venous catheter (external diameter 2.33 mm (7 F), length 30 cm, Teleflex Medical Europe Ltd., Athlone, Ireland) percutaneously into the contralateral common femoral vein for drug administration. We inserted two sheaths percutaneously into the right jugular vein (external diameter 2.83 mm (8.5 F) and 2 mm (6 F), both length 10 cm, Teleflex Medical Europe Ltd.). After surgical cut-down, we positioned a perivascular flow probe (Transonic Flowprobe, Transonic Systems Inc., New York, NY, U. S.) around the left carotid artery in 36 animals and connected it to a transit time flowmeter module (Hugo Sachs Elektronik – Harvard Apparatus GmbH, March-Hugstetten, Germany). We administered Heparin 100 IU/kg bw as a bolus followed by continuous infusion of Heparin 25 IU/kg bw/h to prevent clot formation due to catheterization or cardiac arrest. Then, we introduced a pulmonary artery catheter (external diameter 2.33 mm (7 F), Teleflex Medical Europe Ltd.) through the bigger right jugular sheath under constant monitoring of its pressure readings to ensure pulmonary artery wedge positioning.

Experimental Protocol

After a hands-off period of at least 30 minutes for stabilization, we bedded all swine between the two legs of a V-shaped positioning aid to ensure optimal positioning during the whole period of CA and CPR. We monitored ECG, $etCO_2$, S_pO_2, arterial and central venous blood pressure as well as carotid blood flow continuously throughout the whole experimentation. Following the sampling of baseline conditions, we introduced a bipolar pacing catheter

(external diameter 1.67 mm (5 F), electrode spacing 10 mm, Abbott Medical GmbH, Eschborn, Germany) through the smaller right jugular sheath and applied alternating current (11 V, 0.5 A) for a maximum of 2 seconds (s) in order to induce ventricular fibrillation (VF) in swine randomized to ASCDefib or CONVDefib. When CA was deemed to be confirmed by typical ECG readings and sudden loss of arterial pressure pulsation, we discontinued mechanical ventilation but maintained total intravenous anesthesia in the previously required doses.

After 5 minutes of untreated cardiac arrest, we started CPR with external closed chest compressions applied to the lower sternal half (distance to xyphoid about 5 cm). Seeking a high level of standardization, we used the mechanical chest compression device corpuls cpr (corpuls, Kaufering, Germany) with a custom-made mount for the V-shaped positioning aid, stamp diameter of 80 mm and the following settings: continuous compression mode, compression depth 60 mm, compression rate 100 per minute, duty-cycle 50 % (preset). We resumed ventilation with settings adjusted to the reduced cardiac output: volume-controlled, tidal volume 10 ml/kg bw, PEEP 5 cmH_2O, F_iO_2 100 %, and respiratory rate 16 per minute.

We designed our CPR protocol in accordance to current advanced cardiac life support guidelines [9, 10]: For rhythm checks, CPR was only briefly (< 5 s) interrupted prior to defibrillation or if a marked increase in systolic arterial blood pressure of > 2.67 kPa (> 20 mmHg) or $etCO_2$ of > 0.67 kPa (> 5 mmHg) suggested ROSC. No vasopressors were used before at least three unsuccessful defibrillations.

If the preceding rhythm check confirmed persistent VF, defibrillations (target value for each 4 J/kg bw) were performed after 2, 4, 6 and 8 minutes following the group-specific defibrillation protocol:

ASCDefib

- defibrillations no. 1, 2 and 3 ascending waveforms
- defibrillation no. 4 rectangular waveform (crossover rescue procedure)

CONVDefib

- defibrillation no. 1, 2 and 3 rectangular waveforms
- defibrillation no. 4 ascending waveform (crossover rescue procedure)

In order to control for vasopressor related modulation of ischemia reperfusion damage we assigned animals open-label to either epinephrine 0.01 mg/kg bw (adrenaline, Suprarenin 1 mg/ml, Sanofi-Aventis, Frankfurt, Germany) or vasopressin 0.5 IU/kg bw (Argipressin/Arginin-Vasopressin, Empressin 20 IU/ml, Amomed Pharma GmbH, Wien Austria). In accordance to current guidelines, we administered the chosen vasopressor after the third defibrillation if no obvious signs suggesting ROSC occurred.

Animals that did not show signs of ROSC within 2 minutes after the fourth defibrillation (crossover rescue procedure) were termed “non-survivors” with regard to the present study. If a spontaneous perfusing heart rhythm could be achieved by one out of the four

defibrillations applied, we stopped our CPR efforts and continued hemodynamic measurements for 60 minutes. If cardiac arrhythmia with relevant hypotension occurred, we performed synchronized cardioversion using the corpuls3 as a market approved medical device.
All animals, regardless of CPR outcome, were euthanized after all experimental measurements had been finished by central venous injection of pentobarbital 50 to 100 mg/kg bw (Euthadorm 400 mg/ml, CP-Pharma, Burgdorf, Germany) under continuation of total intravenous anesthesia.

Sham-Operated Control Group

Animals in the sham-operated control group received identical treatment except for induction of VF and CPR. After preparation, we continued total intravenous anesthesia, infusion therapy, monitoring and sampling in these animals for the measured duration of CPR and ROSC in the two intervention groups.

Measurement protocol

Success Rates

In advance, we drew a distinction between initially successful defibrillations and ROSC: We defined those defibrillations as initially successful that led to an organized ECG rhythm that generated measurable blood pressure waveforms for at least 5 s. ROSC was defined as an organized ECG rhythm that generated a systolic blood pressure of at least 8 kPa (60 mmHg) for more than 10 consecutive minutes in accordance to the Utstein-style guidelines for laboratory research on CPR [4]. We defined our primary objective "first shock success" as ROSC after the first defibrillation. We noted the consecutive number of initially successful defibrillations and those leading to persistent ROSC. Furthermore, we evaluated the frequency of re-fibrillation after initially successful defibrillation as well as the frequency of supraventricular and ventricular cardiac arrhythmia after ROSC leading to relevant hypotension.
The conversion rate of the crossover rescue procedure (fourth defibrillation with different waveform) is presented together with the previously administered vasopressor (epinephrine or vasopressin).

Medical Engineering

The data for current and voltage recorded by the prototype defibrillator at 4 kHz was analyzed for mean and peak values of current and voltage. The absolute energy of each defibrillation (formula 1) and the cumulated defibrillation energy for each animal was calculated and is presented in relation to bodyweight. In order to determine the net charge delivered during defibrillation, all measured current values (positive and negative) were summed up and divided by the sample frequency (formula 2). Impedance was calculated for each pair of current and voltage data following Ohm's law and averaged for the whole waveform (formula

3). All electrical properties were examined for their value in predicting initial defibrillation success in a generalized linear model.

Formula 1: $E_{abs} = \sum \left| \frac{V_i \cdot I_i}{4\ \mathrm{kHz}} \right| \quad \left[\frac{\mathrm{V \cdot A}}{Hz} = \mathrm{V \cdot A \cdot s = J} \right]$

Formula 2: $Q_{net} = \frac{\sum I_i}{4\ \mathrm{kHz}} \quad \left[\frac{\mathrm{A}}{\mathrm{Hz}} = \mathrm{A \cdot s = C} \right]$

Formula 3: $R_{avg} = \frac{\sum \frac{V_i}{I_i}}{n} \quad \left[\frac{\mathrm{V}}{\mathrm{A}} = \Omega \right]$

Hemodynamic measurements

We performed transpulmonary thermodilution at baseline and 10 and 60 minutes after ROSC. We computed cardiac index post hoc by dividing thermodilution cardiac output by calculated body surface area [11].

Cardiac index is presented together with heart rate and mean arterial pressure for baseline, 10 and 60 minutes after ROSC.

Blood Samples

For the assessment of myocardial injury, we drew blood at baseline and prior to euthanization via the femoral central venous catheter using serum gel S-Monovettes (SARSTEDT AG & Co., Nümbrecht, Germany). These samples were centrifuged after 30 minutes for clot formation and the pipetted serum quantity was stored at -80 °C for later analyses. All samples were analyzed for troponin T (hsTNT, Elecsys Troponin T hs, cobas e 801, Roche Diagnostics GmbH) in swine that survived 60 minutes after ROSC and in sham-operated animals. We calculated the increase from baseline to final sample for each individual and present the results broken down for number of defibrillations required.

Statistical Analysis

We calculated the sample size ex ante based on our previous experiences [12] for our primary objective first shock success with type I error $\alpha < 5$ %, statistical power $1 - \beta > 80$ % and equal sample sizes in both intervention groups. We considered a "number needed to treat" of 4 to be of clinical relevance. Under these assumptions, the required sample size was 26 for each intervention group.

We sampled the data of ECG, pulse oximetry, $etCO_2$, arterial blood pressure and central venous pressure on the corpuls3 monitor and managed them with corpuls.web ANALYSE (Version 2.0.0.960 (Columbus), corpuls, Kaufering, Germany). We sampled flow data using HSE Haemodyn 1.1.1.202 (Hugo Sachs Elektronik – Harvard Apparatus GmbH, March-Hugstetten, Germany). We merged hemodynamic data from all sources by synchronizing the time stamps for cardiac arrest and subsequently analyzed them using R [13] and RStudio (Version 1.1.453, RStudio Inc., Boston, MA, U. S.). We visualized data with the ggplot2 package [14] for R.

We analyzed frequency distributions like first shock success by Fisher's exact test. To further investigate defibrillation efficacy, we fitted a Cox proportional hazards regression model of ROSC events and plotted the respective Kaplan-Meier curves for ASCDefib and CONVDefib.

We tested all numerical data for normal distribution by Shapiro-Wilk tests and Q-Q-plots. Normally distributed numerical data were compared using two-sided Welch t-tests. Other numerical data were compared using two-sided Mann-Whitney *U* test. A p-value < 0.05 was considered statistically significant and in case of repeated measurement corrected by the Bonferroni method. For consistency, we present all data as median [25 % quartile; 75 % quartile].

We built a generalized linear model to predict initial defibrillation success from electrical properties of the individual waveforms by analyzing all variables by single binomial regression at first. We analyzed energy, mean and peak current, mean and peak voltage, net charge and the waveshape itself. From these, we included variables associated to initial defibrillation success with a p-value < 0.10 into the generalized linear model. We used receiver operating characteristic (ROC) curves to compare the predictive performance of the model.

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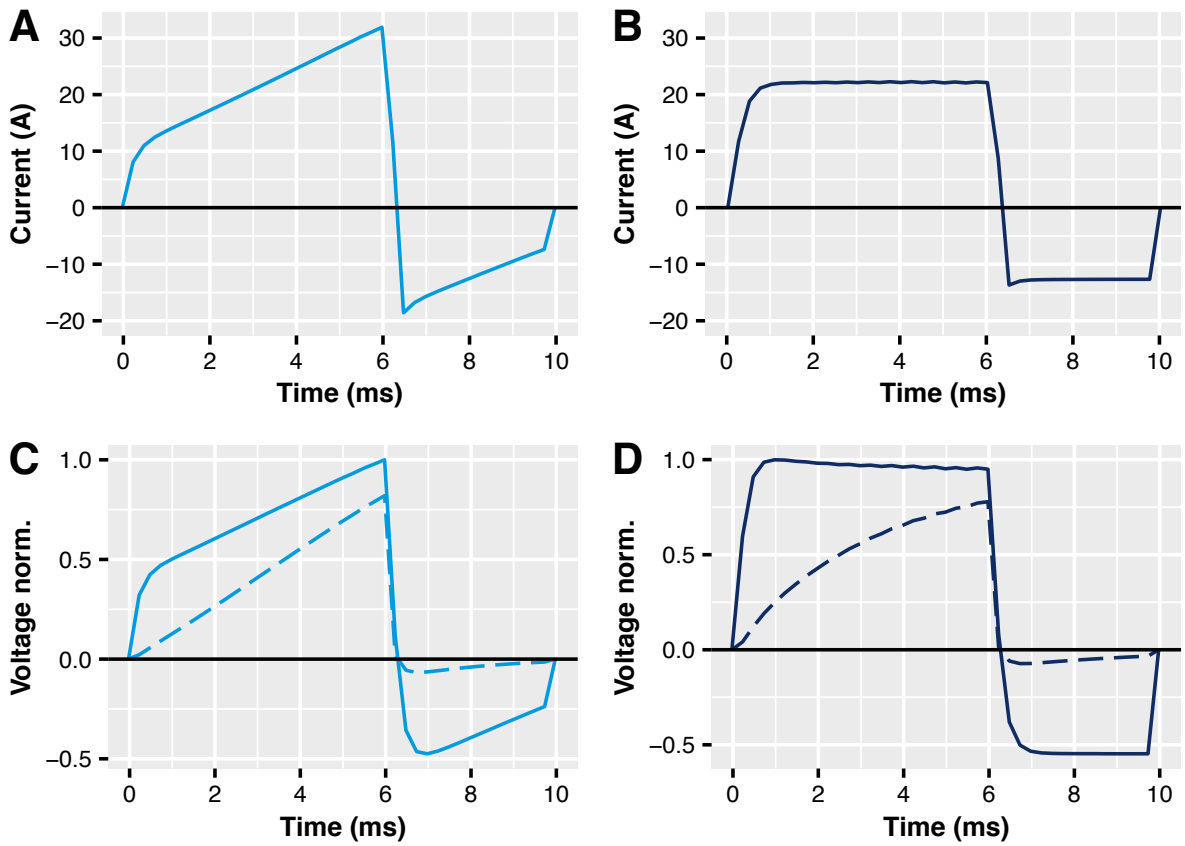
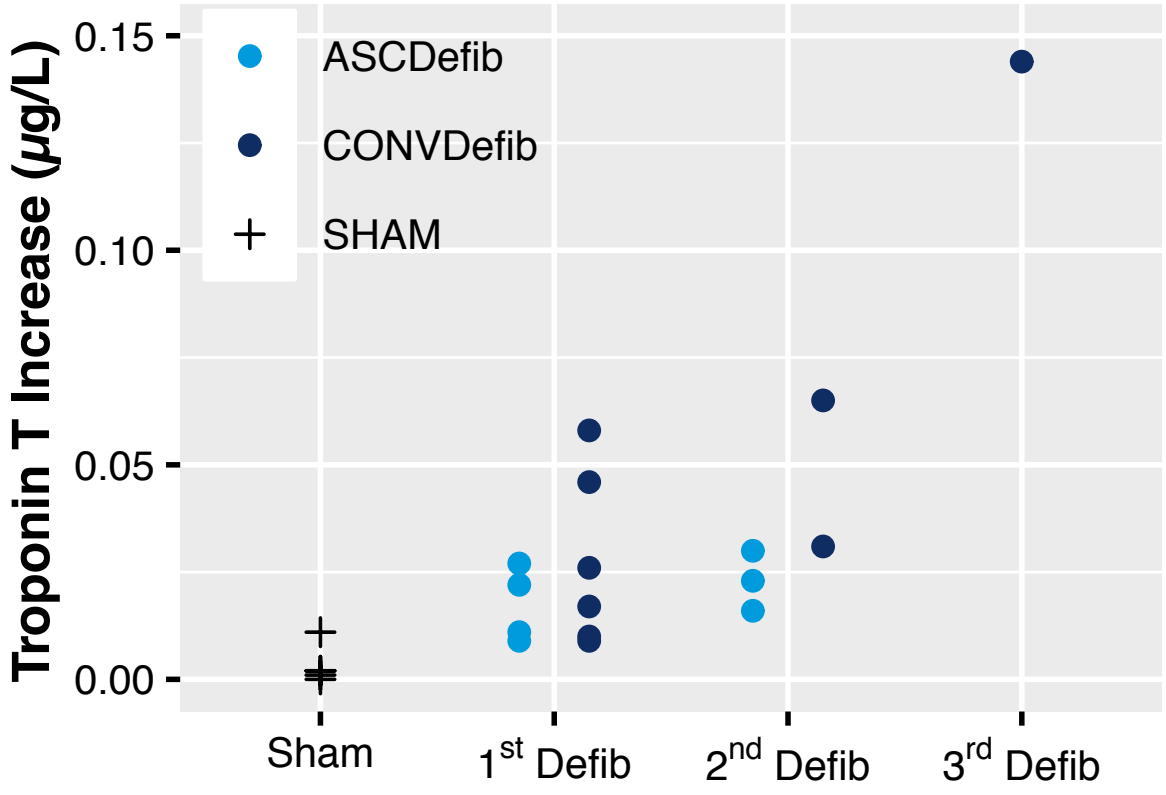
FIGURES



Supplemental Figure 1: Current (ampere) over time (milliseconds) for ascending [A] and rectangular [B] waveforms, as well as voltage (normalized to maximum voltage) over time and the modeled membrane response (dashed line) according to the simplified resistor capacitor model [8] with an assumed membrane time constant of 3.5 ms for ascending [C] and rectangular [D] waveforms. Average of all respective defibrillator discharges.



Supplemental Figure 2: We calculated the increase of Troponin T (µg/L) from baseline to final sample for every individual in the sham control group (n = 5) as well as in the intervention groups ASCDefib and CONVDefib. For swine randomized to an intervention group we present the results broken down by number of defibrillations (Defib) leading to persistent return of spontaneous circulation. (n. s.)

Supplemental Table 1: Success rates for both intervention groups ASCDefib and CONVDefib. The primary objective was first shock success, defined as return of spontaneous circulation (ROSC) after the first defibrillation

	ASCDefib	**CONVDefib**	***p*-value (Fisher's exact test)**
Total number of subjects	26	26	
First shock success	5	7	n. s.
Defibrillation no. 1 – 3 initially successful	12	11	n. s.
Crossover rescue procedure initially successful	2 (rectangular waveform)	1 ascending waveform)	n. s.
ROSC	8	10	n. s.